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Partial block in B lymphocyte development at the transition into the pre-B cell receptor stage in $V_{pre\text{-}B1}$-deficient mice

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Abstract

The surrogate light chain (SL) is composed of two polypeptides, $V_{pre\text{-}B}$ and λ5. In large pre-BII cells the SL chain associates with Ig μ heavy chain (μH) to form the pre-B cell receptor (pre-BCR). In mice there are two $V_{pre\text{-}B}$ genes which are 98% identical within the coding regions. The two genes are co-expressed at the RNA level and encode functional proteins that can assemble with λ5. However, it is not known whether both gene products serve the same function *in vivo*. Here we have established mice that lack the $V_{pre\text{-}B1}$ gene ($V_{preB1}^{-/-}$), but still express the $V_{pre\text{-}B2}$ gene, both as RNA and protein. In $V_{pre\text{-}B1}^{-/-}$ mice, the bone marrow cellularity and the percentage of $B220^+$ cells is normal. However, among the $B220^+$ cells, the percentage of pre-BI cells is increased, and the percentage of pre-BII and immature B cells is slightly decreased, suggesting that the lack of $V_{pre\text{-}B1}$ causes a partial block at the transition from pre-BI to pre-BII cells, i.e. into the pre-BCR stage. The number of cells that produce a functional pre-BCR is thus lower, but the cells that reach this stage are normal as they can be expanded by proliferation and then differentiate into more mature cells. The spleens of $V_{pre\text{-}B1}$ homozygous mutant mice show normal numbers of B and T lymphocytes. Moreover, the Ig loci are allelicly excluded and the homozygous mutant mice respond with normal levels of antigen-specific antibodies to T-dependent antigens. These results demonstrate that $V_{pre\text{-}B2}$ alone is capable of supporting B lymphocyte development in the bone marrow and can give rise to immuno-competent cells in the periphery.

Introduction

The λ5 and $V_{pre\text{-}B}$ genes are expressed early in B lymphocyte development, i.e. in pro-B/pre-BI and large pre-BII cells (1–6). Together they encode the surrogate light chain (SL) chain, which has distinct homologies to conventional Ig chains (7). In pro-/pre-BI cells the SL chain associates with a complex of glycoproteins; however, its function at this stage of development is not yet clear (8). In the large pre-BII cell stage the SL chain associates with the Ig μ heavy (μH) chain and forms the pre-B cell receptor (pre-BCR) (9,10). Experiments in which either the transmembrane part of μH or the λ5 gene has been disrupted show that the pre-BCR affects the expansion of large pre-BII cells into more mature B cell compartments (11,12).

In mice, the λ5 chain is encoded by a single gene, whereas the $V_{pre\text{-}B}$ chain is encoded by two genes (3,13). The two $V_{pre\text{-}B}$ genes are almost identical; only 9 bp difference in the coding regions causing a difference of 4 amino acids between the two polypeptides (3,7). On the mRNA level, expression of the $V_{pre\text{-}B1}$ gene has been detected in all pre-BI and pre-BII bone marrow cells, while $V_{pre\text{-}B2}$ was only found in some of the same pre-BI and pre-BII cells. This, however, might be due to a difference in detection levels (6). Both genes encode functional proteins that can assemble with λ5 when expressed in COS cells (6). Due to the high degree of homology it is not known if both $V_{pre\text{-}B}$ proteins are present in pre-BI and pre-BII cells. In this study we analyze how the lack of a functional $V_{pre\text{-}B1}$ gene affects B lymphocyte development.

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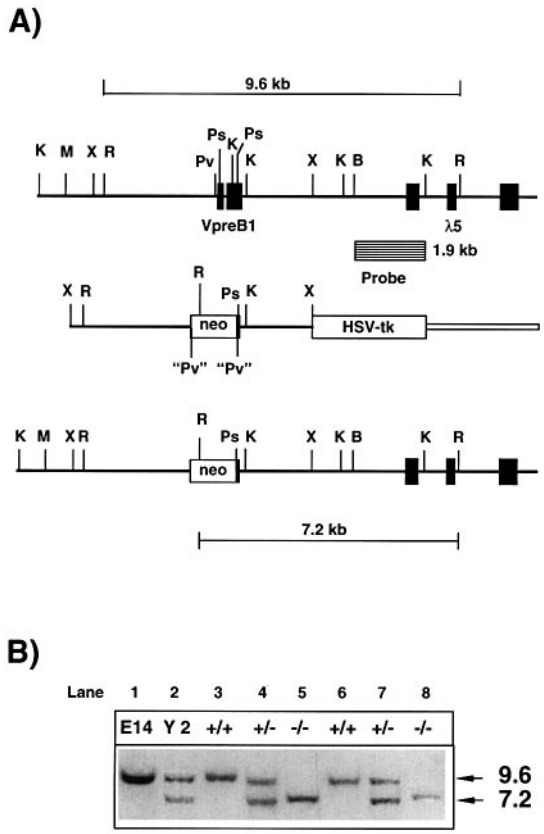


Fig. 1. Homologous recombination of the $V_{pre\text{-}B1}$ loci. (A) Configuration of the $V_{pre\text{-}B1}$/λ5 genomic locus before (top) and after (bottom) homologous recombination with the targeting vector (middle). Homologous integration was detected using *Eco*RI-digested DNA and a probe positioned 3′ outside the vector. The targeted locus would give rise to a band of 7.2 kb in size instead of the 9.6 kb non-targeted allele. Restriction enzyme sites: K, *Kpn*I; M, *Mbo*I; X, *Xba*I; R, *Eco*RI; Pv, *Pvu*II; 'Pv', destroyed *Pvu*II site; Ps, *Pst*I; B, *Bam*HI. (B) Southern blot to distinguish the targeted locus. The genomic DNAs were digested with *Eco*RI. Lanes 1–8; the E14 embryonic stem cell line, the positive Y2 clone, mouse #129 (+/+), mouse #128 (+/–), mouse #127 (–/–), mouse #120 (+/+), mouse #119 (+/–), mouse #118 (–/–). Mice #127–129 are littermates as are #118–120.

Methods

DNA constructs

All DNA constructs were made using conventional DNA techniques (14). The $V_{pre\text{-}B1}$ targeting construct was made in several steps using subcloned fragments from the phage 7pB12 (13). The final construct contained the following in 5′ to 3′ order (Fig. 1): a 3.2 kb *Xba*I–*Pvu*II fragment from the region 5′ of exon I was followed by the *neo* gene from pMC1neopolyA. Downstream of *neo* is an additional 46 bp (from *Pvu*II to *Pst*I) also located in the 5′ untranslated region of $V_{pre\text{-}B1}$. The rest of exon I and most of exon II were removed by deleting the fragment between this *Pst*I site and the *Pst*I site within exon II. The C-terminal 109 bp of exon II is present, as well as the 2.1 kb of 3′ sequence up to the *Xba*I site. This construct therefore contains the sequence for 36 out of 142 amino acids encoded by the $V_{pre\text{-}B1}$ gene. The thymidine kinase gene was inserted downstream of the $V_{pre\text{-}B1}$ sequence (but was not taken advantage of).

Embryonic stem cells and transfections

The procedure has been described before (12). Briefly, the embryonic stem cell line E14 was grown in Iscove's medium supplemented with 2-mercaptoethanol, MEM, antibiotics, LIF (0.1%) and 15% FSC (Boehringer). The LIF was harvested from CHO-LIF cells. Tissue culture plates were gelatinized with 0.1% gelatine after which a layer of G418-resistant embryonic fibroblasts was plated. The fibroblasts were irradiated with 3000 rad before adding the embryonic stem cells. The embryonic stem cells were used at 10^7 cells per transfection event. The cells were transfected by electroporation and after ~36 h selected in 0.2 mg (active) G418/ml. Resistant colonies were picked, expanded and frozen. Parallel cultures, in the absence of feeder cells, were set up for preparation of genomic DNA.

Screening of positive embryonic stem cell clones

Genomic DNA from individual clones were screened by Southern blotting. The DNA was digested with *Kpn*I. The probe used was derived from a region outside the construct. Positive colonies were confirmed by digestion with *Eco*RI using a probe not contained in the targeting construct (Fig. 1; probe, 1.9 kb *Bam*HI–*Kpn*I fragment). Clone Y2, which was later used to establish mice, was further confirmed by several other restriction enzyme digests.

Mice

The Y2 clone was used to establish chimeric mice, using blastocysts from C57Bl/6. The male chimeras were bred with C57Bl/6 females, and heterozygous and homozygous mice were established. After this the mice were interbred. The C57Bl/6 mice were obtained from Bomholtgaard (Ry, Denmark).

Screening tail DNA for genotype

Tail DNA was prepared as described (12). To distinguish between wild-type, heterozygous and homozygous mice we used Southern blotting as described above or a PCR assay in which a mixture of 3 primers (VpB1 sense, VpB1 antisense and *neo* sense) were used. The thermal cycling profile was: 94°C, 20 s; 62°C, 20 s; 72°C, 30 s for 30 cycles. Under these conditions the wild-type allele gives rise to a 430 bp fragment and the disrupted allele yields a 230 bp fragment.

Oligonucleotides

The oligonucleotides used were as follows. *neo* sense: 5′-AAGGCACCTTGGAGGTAATGCAT-3′. HGPRT sense: GCTGGTGAAAAGGACCTCT. HGPRT anti-sense: 5′-CACAGGACTAGAACACCTGC. The $V_{pre\text{-}B1}$, $V_{pre\text{-}B2}$ and λ5 primers have been described before (6).

RNA isolation and RT-PCR

Total RNA from bone marrow cells was isolated using Ultraspec and conditions according to the manufacturer (Ultraspec; Biotec). First-strand cDNAs were synthesized from 1 μg total RNA by reverse transcription with random primers and these cDNAs were used as templates in PCR reactions

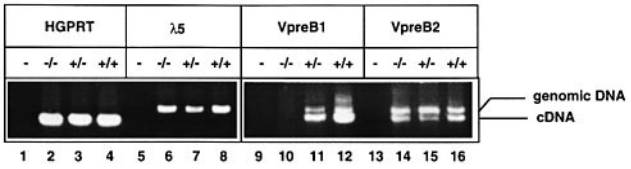


Fig. 2. Lack of $V_{pre\text{-}B1}$ expression in bone marrow cells of $V_{pre\text{-}B1}^{-/-}$ mice. Bone marrow cells from the mice in Fig. 1 were used for RNA preparations. An RT-PCR assay, that distinguishes $V_{pre\text{-}B1}$ from $V_{pre\text{-}B2}$ message, was used to analyze for $V_{pre\text{-}B}$ gene expression (6). The lower band represents RNA (cDNA) while the upper band corresponds to genomic DNA (87 bp larger). The presence of λ5 message was also tested. HGPRT was used as a control for the amount and quality of the cDNA. Lanes 1, 5, 9 and 13: negative control (–); lanes 2, 6, 10 and 14: mouse #118 (–/–); lanes 3, 7, 11 and 15: mouse #119 (+/–); lanes 4, 8, 12 and 16: mouse #120 (+/+).

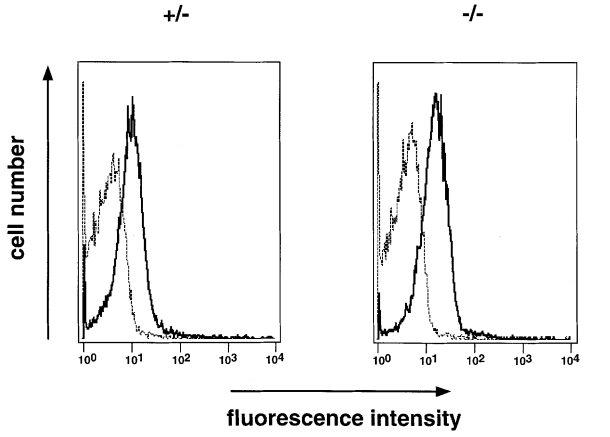


Fig. 3. *In vitro* cultured pre-B cells express $V_{pre\text{-}B2}$ on the cell surface. Total bone marrow from heterozygous and homozygous 7-week-old littermates were cultured on ST2 cells in the presence of IL-7 (15). After 6 days cells were stained with antibodies recognizing CD45R and $V_{pre\text{-}B}$ (VP245) or an isotype-matched control antibody (rat IgG2a) and analyzed by FACS. In the lymphocyte gate 99% of the cells expressed the CD45R marker. The data are presented as a histogram showing cell number and fluorescence intensities of the isotype control (dashed line) and VP245 (solid line) antibody stainings.

(14) using a DNA thermal cycler (Perkin-Elmer, Foster City, CA) with Taq polymerase from Gibco/BRL (Bethesda, MD). Differential amplification of specific $V_{pre\text{-}B1}$ and $V_{pre\text{-}B2}$ transcripts was performed as described (6). Samples were also assayed for level of HGPRT transcripts, as a control for the quality and quantity of RNA. Also λ5 message was analyzed to confirm that it was not affected by the $V_{pre\text{-}B1}$ deletion or by the insertion of the *neo*-resistance gene. The λ5 message was detected as described before (6).

Pre-B cell cultures

Pre-B cells from bone marrow of the indicated mice (Fig. 3) were cultured for 6–7 days as described (15). Briefly, ~25–50×10^3 total bone marrow cells were plated in 24-well plates on γ-irradiated ST2 stromal cells in the presence of 100 U IL-7/ml (16). The cells were cultured in IMDM-supplemented with 2-mercaptoethanol, antibiotics and 5% heat-inactivated FCS.

Flow cytometric analysis

Single-cell suspensions from bone marrow were prepared by flushing out cells from femurs with ice-cold staining buffer (HBSS containing 3% FCS). Spleen, lymph node and thymus cells in single-cell suspensions were stained with the antibodies described below and then analyzed by flow cytometry (FACS) using a FACSort (Becton Dickinson). The cells were gated on the extended lymphocyte gate and then analyzed for reactivity with mAb as described (17,18). Antibodies recognizing the following markers were used: CD45R (B220, FITC-conjugated from PharMingen), CD43, CD25 and IgM (biotinylated from PharMingen), c-kit (19) and $V_{pre\text{-}B}$ (VP245) (8) (the latter antibodies were biotinylated by us), CD4 [phycoerythrin (PE)-conjugated from PharMingen], CD4 (GK1.5, FITC-conjugated by us), CD5 (biotinylated from PharMingen), CD8 (PE-conjugated from PharMingen), CD19 (biotinylated from PharMingen) and IgD (1.19, biotinylated by us). Biotinylated antibodies were revealed by PE–streptavidin (Southern Biotechnology Associates, Birmingham, AL). For the Ig allelic exclusion analysis antibodies recognizing IgM^a (RS3.1, FITC-conjugated by us) and IgM^b (PE-conjugated from PharMingen) allotypes were used.

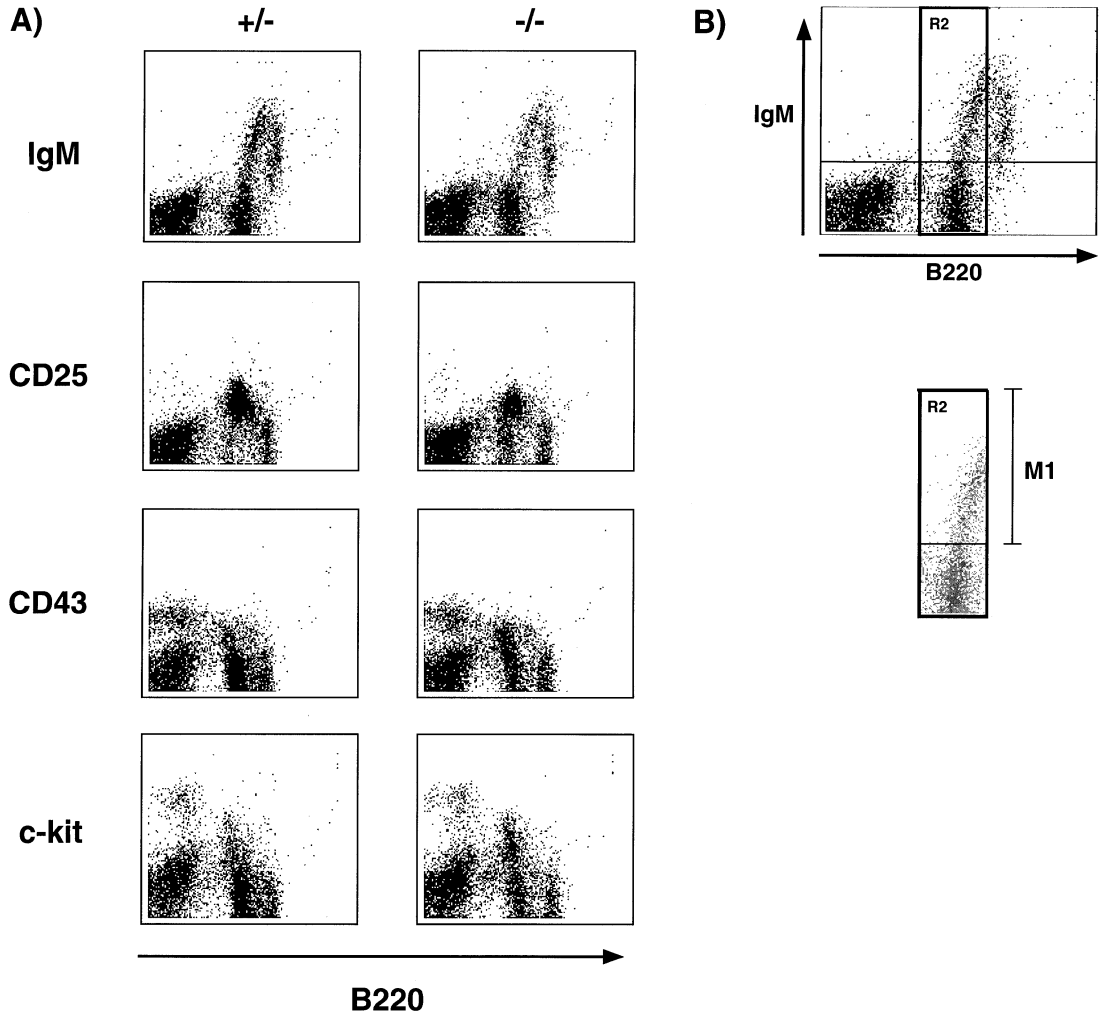
Immunizations and ELISA

Wild-type, heterozygous and homozygous mice (four to five mice in each group) were immunized i.p. with 100 μg of the T-dependent antigen phenyl-oxazolone coupled to ovalbumin as alum precipitate. Two weeks later sera were collected and analyzed for the presence of hapten-specific (plates coated with phenyl-oxazolone coupled to BSA) or carrier-specific (ovalbumin) antibodies in ELISA.

Results

Establishment of $V_{pre\text{-}B1}$-deficient mice

To analyze the role of $V_{pre\text{-}B1}$ and $V_{pre\text{-}B2}$ in B cell development we set out to establish mice lacking the germline $V_{pre\text{-}B1}$ gene. Comparing the restriction enzyme maps of the two $V_{pre\text{-}B}$ genes shows that the homology between the two extends no further than a few hundred base pairs 5′ and 3′ of the coding regions (6). Therefore, a vector that would disrupt the $V_{pre\text{-}B1}$ gene would not be expected to integrate at the $V_{pre\text{-}B2}$ locus at this frequency. A vector to replace the $V_{pre\text{-}B1}$ gene with the gene encoding neomycin resistance was constructed as shown in Fig. 1(A). Embryonic stem cells were transfected and G418-resistant clones screened for homologous recombination. One clone, Y2, out of 352 analyzed was found to carry a disrupted $V_{pre\text{-}B1}$ gene on one allele and was therefore used to establish mice homozygous for the deletion in the $V_{pre\text{-}B1}$ gene ($VpB1^{-/-}$). A Southern blot is shown in Fig. 1(B) in which *Eco*RI-digested DNA from several sources was analyzed by hybridization with the 1.9 kb probe in Fig. 1(A). The E14 embryonic stem cell line shows one band of ~9.6 kb, while the Y2 clone shows two bands, one of which is 9.6 kb and the other is 7.2 kb. The latter band corresponds to the disrupted $V_{pre\text{-}B1}$ allele. In the same figure is shown DNA from individual mice, some of which are wild-type (+/+), heterozygous (+/–) and homozygous (–/–). Thus, the Y2



embryonic stem cell clone gave rise to mice with a deletion in the $V_{pre\text{-}B1}$ gene.

Presence of $V_{pre\text{-}B2}$ but not $V_{pre\text{-}B1}$ RNA

To confirm that the mutation abolished expression of $V_{pre\text{-}B1}$ transcripts, bone marrow RNA was prepared from the mice analyzed in Fig. 1. The RNAs were analyzed in a RT-PCR-based assay which distinguishes between $V_{pre\text{-}B1}$ and $V_{pre\text{-}B2}$ mRNA (6). As seen in Fig. 2, $V_{pre\text{-}B1}$ wild-type (+/+), heterozygous (+/–) and homozygous mutant (–/–) mice all made $V_{pre\text{-}B2}$ mRNA. However, $V_{pre\text{-}B1}$ transcripts could not be detected in $V_{pre\text{-}B1}^{-/-}$ mice, while they were found in both the wild-type and heterozygous mutant mice, as expected. PCR of $V_{pre\text{-}B1}$ or $V_{pre\text{-}B2}$ produced two bands, the larger of which corresponds to either genomic DNA or unspliced RNA (the primers span the 87 bp intron). Note that $V_{pre\text{-}B1}^{-/-}$ mice also lacked this band when $V_{pre\text{-}B1}$, but not $V_{pre\text{-}B2}$ expression was examined (*cf.* Fig. 2, lanes 10 and 14). Since the $V_{pre\text{-}B1}$ sense primer is situated in the region that was deleted upon homologous recombination, this provided additional evidence that this part of the $V_{pre\text{-}B1}$ gene had been deleted. The same cDNA preparations were also analyzed for the presence of λ5 (and HGPRT) message and similar levels were detected in all mice. This demonstrates that expression of the λ5 gene was not affected by the $V_{pre\text{-}B1}$ deletion or by the insertion of the *neo* resistance gene into the $V_{pre\text{-}B1}$ locus which is located 4–5 kb upstream of the λ5 gene. Thus, the targeting vector disrupted the $V_{pre\text{-}B1}$ gene and prevented its expression but did not alter expression of λ5 or $V_{pre\text{-}B2}$.

The $V_{pre\text{-}B2}$ gene encodes a functional protein

Earlier experiments showed that both $V_{pre\text{-}B1}$ and $V_{pre\text{-}B2}$ could associate with λ5 to form a SL chain (6). Since the $V_{pre\text{-}B2}$, but not the $V_{pre\text{-}B1}$, gene was expressed in the mutant mice we investigated if $V_{pre\text{-}B2}$ was present on the cell surface of bone marrow-derived pre-B cells cultured *in vitro* (15). After 6 days in culture on stromal cells, in the presence of IL-7, the cells were analyzed by FACS after staining for expression of B220 (CD45R) versus an antibody (VP245) that recognizes both the $V_{pre\text{-}B1}$ and $V_{pre\text{-}B2}$ proteins or an isotype-matched control antibody (6,8). The cells in the lymphocyte gate were large and 99% stained positive for B220 (data not shown). Figure 3 shows that the VP245 antibody recognized a cell surface bound $V_{pre\text{-}B}$ protein in cells from both $V_{pre\text{-}B1}$ heterozygous and homozygous mutant mice which, in the latter case, corresponds to only $V_{pre\text{-}B2}$. Thus, as suggested earlier

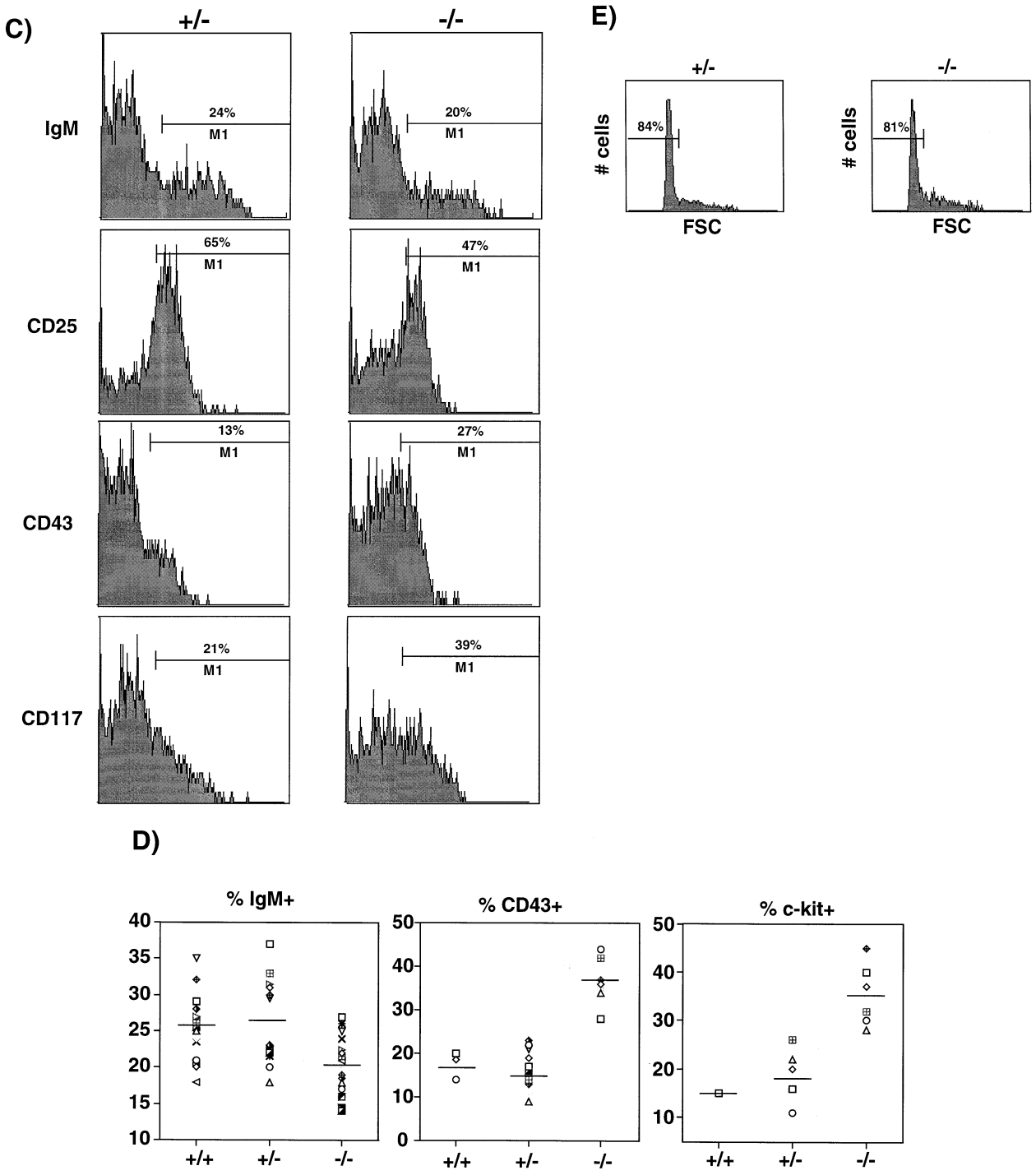


Fig. 4. Partial block in B cell development at the transition into the pre-BCR stage. (A) Expression of CD45R versus IgM, CD25 (IL2-Rα), CD43 and CD117 (c-kit) respectively on bone marrow cells from $V_{pre\text{-}B1}$ heterozygous (+/–) and homozygous (–/–) mutant mice at ~8 weeks of age. Shown is the staining pattern after gating on lymphoid cells. (B) Expression of CD45R versus IgM. The $B220^{lo/int}$ cells were selected as gate R2 and the percentage of IgM^+ cells within this gate determined; M1. (C) The cells in (A) were analyzed by gate R2 ($B220^{lo/int}$) to determine M1. The data are displayed as histograms showing the M1 values, i.e. percentage of cells in gate R2 being positive for IgM, CD25, CD43 or CD117. (D) Between one and 10 mice per group (in several separate experiments) were analyzed as in (C). The M1 values for IgM, CD43 and CD117 are shown. Student's *t*-test on the decrease in percentage of IgM^+ cells gave a $P < 0.1$, i.e. highly significant. The CD117 staining has been confirmed with more wild-type and homozygous mutant mice using another CD117 antibody. (E) The $CD25^+$ cells in (A) were gated and the percentage of small (FSC) cells determined.

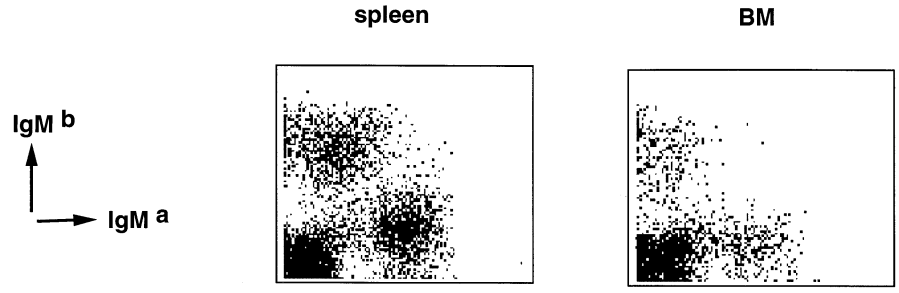


Fig. 5. Allelic exclusion of the IgH loci. Cells from spleen (left panel) and bone marrow (right panel) were stained with markers specific for IgM^a versus IgM^b allotype. Cells from $V_{pre\text{-}B1}$ homozygous mutant, Ig allotype heterozygous, mice are shown.

(6), the $V_{pre\text{-}B2}$ gene encodes a functional protein that can be expressed on the cell surface of pre-B cells.

Partial block at the transition into the pre-BCR stage

To determine whether the failure to express $V_{pre\text{-}B1}$ had any effect on B cell development we first analyzed the spleen of $V_{pre\text{-}B1}^{-/-}$ mice. Here, we found that the total number of cells, and the percentages of cells expressing B220, IgM, CD8 and CD4 were about normal, suggesting that the peripheral B and T cell compartments were not affected (data not shown). We then analyzed B220 and IgM expressing B cells in lymph nodes which showed the same pattern in all three genotypes (data not shown). Lastly, analysis of thymus demonstrated a normal pattern of CD4 and CD8 positive cells (data not shown). Thus, spleen, lymph node and thymus in the $V_{pre\text{-}B1}$-deficient mice were normal in terms of B and T lymphocytes.

Since SL chain is expressed and functions specifically at early stages of B cell development, bone marrow cells were analyzed by different means. The cells were counted and the number of nucleated cells in homozygous mutant mice was found to be similar to that of wild-type and heterozygous mutant mice. Thereafter cells were analyzed for expression of B220 in combination with either IgM, CD25, CD43 or CD117 (c-kit). The differential expression of these markers is characteristic of their differentiation stage (17,18). The percentage of cells expressing B220 was found to be ~50% in all three genotypes. As shown in Fig. 4(A), the homozygous mutant mice stained positive for B220 in combination with all the above-mentioned markers. However, the percentage of cells in the various compartments differed in $V_{pre\text{-}B1}^{-/-}$ mice as compared to heterozygous and wild-type mice: the $B220^+$ c-kit^+ and $B220^+$ $CD43^+$ compartments were increased, while the $B220^+$ $CD25^+$ and $B220^+$ IgM^+ compartments were slightly decreased.

To more closely analyze these differences, bone marrow cells that expressed low to intermediate levels of B220 ($B220^{lo/int}$) were analyzed further ($B220^{high}$ cells are recirculating cells). The $B220^{lo/int}$ cells were gated (Fig. 4B, gate R2) and the percentage of cells within this gate that expressed the above markers determined (Fig. 4B, M1). As shown in the histograms in Fig. 4(C) and the summary in Fig. 4(D), the percentage of $B220^{lo/int}$ IgM^+ cells was slightly decreased in $V_{pre\text{-}B1}^{-/-}$ mice. The same result was also observed for $B220^{lo/int}$ $CD25^+$ cells (Fig. 4C and data not shown). In contrast, the percentage of $B220^{lo/int}$ $CD117^+$ cells was ~2-fold higher in homozygous as compared to heterozygous mutant mice. This was also the case for $B220^{lo/int}$ $CD43^+$ cells. A summary of several experiments, which also included wild-type mice, is shown in Fig. 4(D). Since the number of bone marrow cells in all three genotypes was about the same, the increase in the percentage of $B220^{lo/int}$ $CD117^+$ cells represented an increase in the actual number of $B220^{lo/int}$ $CD117^+$ cells. The size distribution among the cells that expressed either CD117, CD43, CD25 or IgM was similar among the three genotypes, suggesting that the lack of $V_{pre\text{-}B1}$ did not affect the expression of these markers *per se* (Fig. 4E and data not shown). Thus, the number of bone marrow cells expressing $B220^{lo/int}$ in combination with CD117 and CD43 was enriched for by a factor of 2, while $B220^{lo/int}$ $CD25^+$ and $B220^{lo/int}$ IgM^+ cells were each decreased by ~20% in $V_{pre\text{-}B1}^{-/-}$ mice.

Pre-BI cells express B220, CD117 and CD43 but not CD25; pre-BII cells are $B220^+$ $CD25^+$ but lack c-kit and CD43. The CD25 marker is not found on more differentiated cells, i.e. immature and mature B cells (18). Large pre-BII cells that express a functional pre-BCR are expanded by proliferation and, upon differentiation, give rise to small pre-BII cells. Among the $B220^{lo/int}$ $CD25^+$ cells ~20 and 80% constituted large and small cells respectively in all three genotypes (Fig. 4E and data not shown). This suggested that cells with a functional pre-BCR were expanded also in the marrow of $V_{pre\text{-}B1}$ homozygous mutant mice. Thus, bone marrow from $V_{pre\text{-}B1}^{-/-}$ mice exhibited an enrichment in pre-BI cells ($B220^{lo/int}$ $CD117^+$ $CD43^+$) and a reduction in large pre-BII cells ($B220^{lo/int}$ $CD25^+$) suggesting that the lack of $V_{pre\text{-}B1}$ affected B lymphocyte development at the transition into the pre-BCR stage.

Ig allelic exclusion in V_{preB1}-deficient mice

To determine if the $V_{pre\text{-}B2}$-containing pre-BCR was functional in terms of allelic exclusion of the Ig loci, we analyzed spleen and bone marrow cells by FACS for the presence of both Ig allotypes in Ig^a/Ig^b heterozygous mice. As shown in Fig. 5, >98% of all $sIgM^+$ B cells of bone marrow and of spleen from $V_{preB1}^{-/-}$ Ig allotype heterozygous mice expressed either one or the other, but not both Ig allotypes on one cell. Thus, a defective $V_{pre\text{-}B1}$ gene did not affect Ig allelic exclusion.

Normal immune response against T-dependent antigens

We next examined the ability of $V_{pre\text{-}B1}$-deficient mice to mount a T-dependent antigen response by immunizing with the

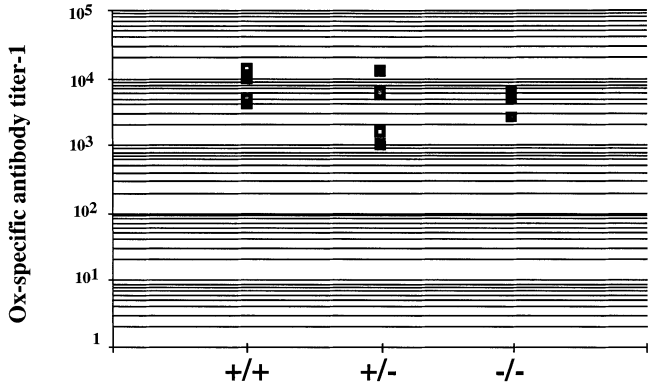


Fig. 6. Normal immune response to a T-dependent antigen. $V_{pre\text{-}B1}$ wild-type (+/+), heterozygous (+/–) and homozygous (–/–) mutant mice were immunized (i.p.) with 100 μg alum precipitate of the T-dependent antigen phenyl-oxazolone coupled to ovalbumin as a carrier. Two weeks later sera were taken and analyzed for hapten (Ox)-specific antibodies in ELISA. Each group contained four or five mice. Shown is the titer of each serum at 50% of the plateau level. Sera from non-immunized mice: <1.

hapten phenyl-oxazolone coupled to the carrier ovalbumin. Two weeks after immunization, serum were tested in ELISA for the presence of hapten- and carrier-specific antibodies. As shown in Fig. 6, all mice showed a good response to the hapten, independent of the status of the $V_{pre\text{-}B1}$ loci. The response to the ovalbumin carrier was also normal in all mice (data not shown). Therefore, the absence of $V_{pre\text{-}B1}$ did not alter the immune response to T-dependent antigens.

Discussion

Our aim here was to evaluate the role in B lymphocyte development of one of the two $V_{pre\text{-}B}$ genes present in the mouse genome, i.e. the $V_{pre\text{-}B1}$ gene. We find that disruption of the $V_{pre\text{-}B1}$ gene causes a partial block in B cell development at the transition into the pre-BCR stage as defined by an increase in the number of pre-BI cells and a slight decrease in the number of large pre-BII cells. However, the number of mature B cells in the periphery was not affected. Moreover, allelic exclusion of IgH gene expression, in the central and peripheral B cell compartments, is functional as are T cell-dependent immune responses. This demonstrates that both $V_{pre\text{-}B}$ gene products are functional and involved in the formation of the pre-BCR. It also shows that $V_{pre\text{-}B2}$ alone is sufficient, but not as efficient as the two genes together, in supporting B lymphocyte development.

We showed earlier that the expression pattern at the RNA level of the two $V_{pre\text{-}B}$ genes (and λ5) is similar in fetal liver cells and in pro-B/pre-BI and large pre-BII bone marrow cells. Single-cell RT-PCR of the latter two populations detected $V_{pre\text{-}B1}$ (and λ5) mRNA in all cells, but $V_{pre\text{-}B2}$ transcripts were found in only a portion (30%) of the same cells (6). This discrepancy was proposed to be due to a difference in sensitivity of the primer pairs used in the assays, a hypothesis which is supported by our results herein: the number of large pre-BII cells in $V_{pre\text{-}B1}^{-/-}$ mice is too high to be derived from only one-third of the normal numbers of pre-BI cells. In transfection experiments, both $V_{pre\text{-}B}$ gene products have been shown to assemble with the λ5 protein. However, it is not known if they do so *in vivo* since there is no antibody described that distinguishes the two $V_{pre\text{-}B}$ proteins (6,20). Our results here demonstrate that the $V_{pre\text{-}B2}$ gene product can be incorporated in both the pro-B and pre-B cell receptors of pro-B/pre-BI and large pre-BII bone marrow cells. Furthermore, $V_{pre\text{-}B2}$ can function in all steps of development, especially in the pre-BCR-dependent proliferative expansion of large pre-BII cells. If the pre-BCR is also involved in allelic exclusion of the IgH chain gene expression, then $V_{pre\text{-}B2}$ in pre-BCR can also do that.

The importance of the membrane form of the μH chain and the λ5 gene product at the pre-BCR stage was demonstrated earlier in mice lacking the respective gene product (11,12). Here we show that also the $V_{pre\text{-}B1}$ gene product is important at the pre-BCR stage since the lack of $V_{pre\text{-}B1}$ causes an enrichment of cells at the pre-BI stage, i.e. a partial block at the transition into the pre-BCR stage. It has recently been shown that not all Ig μH chains made from productively VDJ_H-rearranged IgH loci can pair with the SL chain (21). Cells expressing such non-pairing μH chains cannot form a pre-BCR and do not participate in the proliferative expansion of large pre-BII cells. Hence, cells expressing these μH chains do not appear in the subsequent pre-B and B cell repertoires. Some of the inability to pair may well be due to a structural incompatibility of a V_H domain in interactions with $V_{pre\text{-}B}$. If so, then pairing to $V_{pre\text{-}B1}$ and $V_{pre\text{-}B2}$ may differ since some of the nucleotide differences between the two $V_{pre\text{-}B}$ genes encode amino acid replacements. The V_H repertoires of normal and $V_{pre\text{-}B1}^{-/-}$ mice may, therefore, differ. This will be investigated in future experiments.

The cells that progress through the pre-BCR stage in $V_{pre\text{-}B1}^{-/-}$ mice seem to be normal in that they are expanded by proliferation, and the number of more mature cells is just slightly decreased as compared to heterozygous mutant and wild-type mice. This did not significantly affect the number of B cells detected in the periphery. This is in agreement with experiments that showed that a minor decrease in the production of pre-B cells (here pre-BII) in the marrow did not affect the number of B cells in the periphery, while a major decrease in pre-B cells did (22). Such a minor decrease in pre-B cells could be envisioned as the situation in $V_{pre\text{-}B1}$-deficient mice while a major (20 to 40-fold) reduction in pre-B(II) cells is compatible with that found in mice lacking λ5 that also have a significant decrease in the number of peripheral B cells.

Our data herein shows that mice expressing only one $V_{pre\text{-}B}$ gene ($V_{pre\text{-}B2}$) are immunocompetent and give rise to both hapten- and carrier-specific antibodies when challenged with T cell-dependent antigens. Thus, in mice, as is the case in humans, one $V_{pre\text{-}B}$ gene is sufficient, if not as efficient.

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Abbreviations

μH	Ig μ heavy chain
pre-BCR	pre-B cell receptor
SL	surrogate light chain
$V_{preB1}^{-/-}$	$V_{pre\text{-}B1}$ homozygous mutant mice

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